

# メプロニル(バシタック(R))の湛水土壤条件下における容器内での分解

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# Degradation of Mepronil (Basitac®)\* in Soils under Submerged Conditions in the Laboratory

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The degradation of [aniline-U-<sup>14</sup>C]mepronil in a volcanic ash soil (Aso) and an alluvial soil (Kikugawa) was studied under submerged conditions in the laboratory. After incubation of the soil containing <sup>14</sup>C-mepronil at 1.6 ppm in a biometric flask in the dark at 29°C for 80 days, the soil was sequentially extracted with methanol/water (2/1) and acetonitrile (extractable) and the residue (bound) was extracted with dichloromethane after treatment with 12 N-NaOH/methanol (1/3) at 70°C for 2 hr. Mepronil was the major constituent in both extracts. Its half-life was 46 and 50.5 days in Aso and Kikugawa soils, respectively, but counting mepronil bound in soils, the half-life extended to 117 and 120 days, respectively. The degradation products were tightly bound on soil particles and the extractable amounts were small. Four of the 7 products were identified as 1-hydroxy-3-oxo-2-(3'-isopropoxy-phenyl)isoindoline, 3'-isopropoxy-2-hydroxymethylbenzanilide, 3'-hydroxy-2-methylbenzanilide and 3'-(1-hydroxymethylethoxy)-2-methylbenzanilide, indicating that hydroxylation occurs on the three positions of the mepronil molecule.

## INTRODUCTION

Mepronil or Basitac® (code No. BI-2459, 3'-isopropoxy-2-methylbenzanilide), developed by Kumiai Chemical Industry Co., Ltd., has a strong fungicidal activity especially against rice sheath blight fungus (*Rhizoctonia solani* Kuhn),<sup>1)</sup> a lower mammalian toxicity,<sup>2)</sup> and adequate stability under environmental conditions. The present paper is concerned with the fate of <sup>14</sup>C-mepronil in two types of soil under submerged conditions in the laboratory.

## MATERIALS AND METHODS

### 1. Chemicals

<sup>14</sup>C-Mepronil labelled uniformly at anilining (specific activity, 2.72 mCi/mmol; radiochemical purity, more than 99%), was sup-

plied by Professor Shigeo Baba of the Tokyo College of Pharmacy (Hachioji, Tokyo). Authentic compounds for the possible conversion products were prepared in the Chemical Research Institute, Kumiai Chemical Co. (100 Shibukawa, Shimizu, Shizuoka 424, Japan).

Table 1 shows the structures and *R<sub>f</sub>* values on thin-layer chromatography (*t<sub>lc</sub>*) of mepronil and related compounds. The properties of the reference compounds are as follows: Compound I, mp 92-93°C. MS *m/e* 269 (M<sup>+</sup>), 227, 210, 119 (base peak). Compound II, bp 122°C/4.5 mmHg. Compound III, mp 143-144°C. NMR (CD<sub>3</sub>COCD<sub>3</sub>) δ : 1.30 (6 H, d *J*=6 Hz), 4.33-4.82 (1 H, m *J*=6 Hz), 5.73 (1 H, d *J*=10 Hz), 6.48 (1 H, d *J*=10 Hz), 6.60-8.80 (8 H, m arom.); MS *m/e* 283 (M<sup>+</sup>, base peak), 267, 241, 225, 213, 197, 133, 105; IR ν cm<sup>-1</sup> (KBr) : 3325 (-OH), 2960, 2900 (-CH<sub>2</sub>-), 1660 (-CO-). Compound IV, mp 140-142°C. IR ν cm<sup>-1</sup> (KBr) : 3420, 3230 (-OH, -NH-), 1650, 1520 (-CONH-), 1600, 1490 (phenyl). Compound V, bp 251°C. Compound VI, mp 165-166°C. NMR (CD<sub>3</sub>COCD<sub>3</sub>) δ : 1.32 (6 H, d *J*=6 Hz),

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Table 1 *R<sub>f</sub>* values of mepronil and its related compounds.

No.	Compounds	Solvent systems*		
		A	B	C
I	Mepronil: 3'-isopropoxy-2-methylbenzanilide	0.79	0.77	0.87
II	3-Isopropoxyaniline	0.70	0.68	0.68
III	1-Hydroxy-3-oxo-2-(3'-isopropoxyphenyl)isoindoline	0.70	0.53	0.56
IV	3'-Methoxy-2-methylbenzanilide	0.65	0.70	0.85
V	3-Methoxyaniline ( <i>m</i> -anisidine)	0.51	0.63	0.58
VI	3'-Isopropoxy-4'-hydroxy-2-methylbenzanilide	0.65	0.48	0.72
VII	3'-Isopropoxy-2-hydroxymethylbenzanilide	0.45	0.27	0.49
VIII	3'-Isopropoxy-4-hydroxyaniline	0.34	0.29	0.30
IX	3'-Hydroxy-2-methylbenzanilide	0.36	0.19	0.40
X	3'-(1-Hydroxymethylethoxy)-2-methylbenzanilide	0.32	0.22	0.25
XI	3-Hydroxyaniline	0.22	0.18	0.22
XII	3',4'-Dihydroxy-2-methylbenzanilide	0.18	0.11	—
XIII	3'-(1-Carboxyethoxy)-2-methylbenzanilide	0.08	0.04	0.03

\* A: *n*-hexane/dioxane (2/1), B: benzene/methanol (19/1), C: chloroform/ethyl acetate (5/1).

2.43 (3 H, s), 4.60 (1 H, m  $J=6$  Hz), 6.66–7.66 (8 H, m), 9.13 (1 H, b.s.); MS *m/e* 285 ( $M^+$ ), 243, 119 (base peak). Compound VII, mp 116–117°C, NMR ( $CD_3COCD_3$ )  $\delta$ : 1.30 (6 H, d  $J=6$  Hz), 4.15–5.33 (3 H, m  $J=6$  Hz), 6.33–8.00 (9 H, m arom.  $-CH_2OH$ ), 9.30–10.33 (1 H b.s.). Compound VIII, mp 95–96°C. IR  $\nu$   $cm^{-1}$  (KBr): 3400, 3210 ( $-NH_2$ ), 3090 ( $-OH$ ). Compound IX, mp 173–176°C. IR  $\nu$   $cm^{-1}$  (KBr): 3380, 3150 ( $-OH$ ,  $-NH-$ ), 1650, 1540 ( $-CONH-$ ), 1600, 1490 (phenyl). Compound X, bp 198–201°C/0.002 mmHg (mp 32–33°C), NMR ( $CD_3COCD_3$ )  $\delta$ : 1.23 (3 H, d  $J=6$  Hz), 2.33 (3 H, s), 2.90 (1 H, b.s.), 3.63 (2 H d), 4.45 (1 H, d  $J=6$  Hz), 6.66 (1 H, m), 7.26 (7 H, m arom.), 9.30 (1 H, b.s.). Compound XI, mp 122–123°C. Compound XII, mp 93–95°C. NMR ( $CD_3COCD_3$ )  $\delta$ : 2.43 (3 H, s), 3.00 (1 H, b.s.), 6.73–7.66 (7 H, m arom.), 7.83 (1 H, b.s.), 9.10 (1 H, b.s.). Compound XIII, mp 47–48°C. NMR ( $CD_3COCD_3$ )  $\delta$ : 1.60 (3 H, d  $J=6$  Hz), 2.40 (3 H, s), 4.87 (1 H, m

$J=6$  Hz), 6.75–7.67 (9 H, m  $-OH$ , arom.), 9.33 (1 H, d b.s.); IR  $\nu$   $cm^{-1}$  (KBr): 3400–2200 ( $-COOH$ ,  $-NH-$ ), 1720 ( $-COOH$ ), 1640, 1530 ( $-CONH-$ ), 1590, 1490 (phenyl).

## 2. Soil Samples and Treatment of $^{14}C$ -Mepronil

Aso soil, a mineral soil with allophane clay mineral, was obtained from Kumamoto Prefectural Agricultural Experiment Station. Kikugawa soil is a mineral soil with kaoline clay mineral from the experimental farm of Life Science Research Institute, Kumiai Chemical Co. in Shizuoka Pref. The properties of the two soils are given in Table 2.

The undried soils were crushed and put through a 5 mm sieve. Thirty g (oven dried weight basis) of each type of soil was placed in a 300 ml of biometric flask<sup>5,9)</sup> and water added to a depth of 1 cm. The 2 mouths of the flask were covered with aluminum foil, and the soil was preincubated at  $29 \pm 1^\circ C$  in

Table 2 Properties of soil samples used.

Sample site	Texture*	Clay <sup>3)</sup> mineral	Clay content (%)	pH ( $H_2O$ )	pH (KCl)	Total-C** (%)	C.E.C <sup>4)</sup> ( <i>m.e.</i> /100 g)	Maximum water holding capacity (%)
Aso	sandy loam	allophane	7.3	6.85	6.05	1.47	22.7	92.2
Kikugawa	clay loam	kaolinite	46.5	5.77	4.67	1.65	26.6	76.5

\* The international method, \*\* using a C-N corder (Yanagimoto).

the dark for one week. Mepronil is applied at the rate of 4 kg of 3% dust per 10 ares in paddy fields. Accordingly, 1.6 ppm of  $^{14}\text{C}$ -mepronil on dry soil basis (25.4  $\mu\text{g}$  of labelled and 22.5  $\mu\text{g}$  of unlabelled dissolved in 0.5 ml of acetone) was applied dropwise on the submerged soils in the biometric flask and thoroughly mixed. In the side room of the biometric flask was placed 3 ml of 12.5 N-NaOH solution for the adsorption of  $^{14}\text{CO}_2$  liberated from the soil and an Ascarite<sup>®</sup> tube was set to shut off the  $\text{CO}_2$  from the outside. Soils treated with mepronil were incubated<sup>7)</sup> at  $29 \pm 1^\circ\text{C}$  in the dark for 80 days.

### 3. Trapping of $^{14}\text{CO}_2$ and Volatile Organic Materials<sup>8)</sup>

After incubation for the prescribed period, the side room of the biometric flask was connected with two traps. Air was passed by suction using an aspirator, first through 20 ml of toluene to trap volatile organic materials and then through 20 ml of monoethanolamine to trap  $^{14}\text{CO}_2$ . The NaOH solution in the side room was diluted to 10 ml with water.

### 4. Extraction and Separation of Radioactive Compounds

The soil was transferred to a polyethylene-centrifuge tube, and 60 ml of methanol/water (2/1) solution was added. The tube was shaken vigorously for one min and centrifuged for 20 min, and the supernatant was obtained by decantation. The soil residue was reextracted two more times, each with 40 ml of the methanol/water solution by the same procedure. The methanol/water extracts were combined (F-1). The residual soil was then extracted three times, each with 30 ml of acetonitrile and centrifuged as described above to get the extract (F-4). Then, 200 ml of 12 N-NaOH/methanol (1/3) was added to the residue. The tube was capped tightly and warmed at  $70^\circ\text{C}$  for 2 hr. After cooling, 5.0 g of celite 545 were added to the tube and shaken vigorously. The extract (F-5) and the residual soil (F-8) were obtained by filtration. After removing most of the methanol under reduced pressure, each of F-1 and F-5, was extracted with 100 ml of dichloromethane. F-1 gave the extract (F-2) and the aqueous layer (F-3), and F-5 gave

the extract (F-6) and the aqueous layer (F-7). *Tlc* separation was performed for each extract and the aqueous layer. The residual soil (F-8) was air-dried.

### 5. Thin-layer Chromatography and Autoradiography

*Tlc* was performed on silica gel chromatoplate (Merk 60, F<sub>254</sub> precoated, 0.25 mm thickness,  $20 \times 20$  cm). The plate was developed mainly in a two-dimensional way, first with *n*-hexane/dioxane (2/1) and secondly with benzene/methanol (19/1). After development, the *tlc* plate was contacted with X-ray film (Konishiroku Photo Ind. X ray film, JX ( $10 \times 12$ )  $25.4 \times 30.5$  cm) for 7 days to make the autoradiogram. Spots of reference compounds were detected under ultraviolet lamp (254 nm).

### 6. Radioactivity Measurement

One ml aliquot of each solution was transferred into a vial and dissolved with 12 ml of hydrophilic scintillator (DPO 4.0 g, POPOP 0.4 g, naphthalene 100 g, toluene 150 ml, ethyl cellosolve 100 ml, and dioxane 750 ml, to make 1000 ml) and radioassayed using a liquid scintillation spectrometer (Aloka Model LSC-502). Each spot on a *tlc* plate was scraped into a vial, added with 12 ml of liquid scintillator, and radioassayed. The residual soil (F-8, 200 mg) was oxidized by using a combustion system (Aloka Model ASC-112), and the  $^{14}\text{CO}_2$  evolved was trapped with 6 ml of monoethanolamine/methanol (1/1); 12 ml of toluene base scintillator [DPO 10 g, POPOP 0.5 g dissolved in 1000 ml of toluene/methanol (7/5)] was added for radioassay.

### 7. Identification of Degradation Products

The spot on the *tlc* plate was scraped, extracted with methanol, added with the authentic compound, and developed on the *tlc* plate for cochromatography. When needed, a product was methylated with diazomethane or reduced with sodium borohydride, and subjected to *tlc*.

## RESULTS AND DISCUSSION

### 1. Radioactivity Distribution among Extract Fractions

The radioactive residue in the two types of

Table 3 Change in radioactivity of the fractions from two soils treated with [aniline-U-<sup>14</sup>C]mepronil during incubation.

Fraction No.	Days	% of applied radiocarbon					
		0	5	10	20	40	80
Volcanic ash soil (Aso)							
F-1	97.7±2.3	61.1±2.0	62.9±1.5	42.6±2.7	28.1±2.7	11.7±0.8	
F-2		59.6±1.4	62.5±2.4	42.2±1.8	25.1±1.3	11.1±0.6	
F-4	5.5±0.5	24.1±3.8	23.1±1.1	21.7±6.4	32.8±3.7	27.4±2.9	
*Extractable	103.2±1.7	85.3±1.8	86.0±0.7	64.3±7.8	60.9±4.1	39.1±2.3	
**Bound residue		14.7±1.8	14.0±0.7	35.7±7.8	39.1±4.1	60.9±2.3	
F-5	2.2±0.3	16.0±0.9	16.1±0.5	33.2±2.0	27.4±1.3	32.3±0.6	
F-6		12.0±0.8	10.8±0.7	24.0±1.5	23.5±2.2	26.0±2.0	
F-8		0	2.2±0.4	5.5±2.1	12.3±1.8	24.6±0.4	
Alluvial soil (Kikugawa)							
F-1	87.8±15.5	66.6±1.4	60.6±0.7	66.1±3.5	29.0±1.3	17.2±2.3	
F-2		67.2±1.1	56.7±3.5	69.6±1.0	27.2±1.6	12.6±3.7	
F-4	20.1±8.8	31.5±0.9	26.1±1.9	15.2±0.4	37.8±3.7	23.1±0.6	
*Extractable	107.8±6.7	98.1±1.0	86.7±2.0	81.3±3.9	66.8±4.6	40.3±2.6	
**Bound residue		1.9±1.0	13.3±2.0	18.7±3.9	33.2±4.6	59.7±2.6	
F-5		7.8±0.8	18.2±0.8	18.2±1.0	21.9±1.1	34.5±0.8	
F-6		4.8±0.7	15.7±0.9	15.0±2.9	17.9±0.8	27.2±2.1	
F-8		0	0.9±0.2	1.9±1.2	12.3±1.5	20.3±5.3	

\*: F-1+F-4, \*\*: applied radiocarbon-extractable. Values mean ±S.D. of triplicate experiments.

soil was fractionated into extractable fraction and bound residue fraction. A soil bound residue may be defined as the unextractable pesticide residue after exhaustive sequential extraction with nonpolar organic and polar solvents.<sup>9)</sup> Accordingly, many extract systems were examined. Extraction with methanol/water followed by acetonitrile gave the best result in terms of radioactivity extracted from the two different soils. The amounts of <sup>14</sup>CO<sub>2</sub> and volatile materials increased gradually with time. However, their amounts were less than 0.1% during the experimental period and were thus ignored. The combined radioactivity of methanol/water (F-1) and acetonitrile (F-4) extracts constitutes "extractable." The residue from the above extractions, or the combined radioactivity of 12 N-NaOH/methanol (F-5) extract and <sup>14</sup>CO<sub>2</sub> from the residual soil (F-8) constitutes "bound." One hour after the application of <sup>14</sup>C-mepronil, the applied radioactivity was quantitatively recovered from the two soils. Changes in the radioactivity of the extract fractions in Aso and Kikugawa soils during incubation are shown in Table 3. In general, the radioactivity distribution among the fractions did not greatly

differ in the two soils. The discrepancy in theoretical and observed values for "bound" in Table 3 may be due to the effect of ammonia produced by hydrolysis of acetonitrile in F-5 on counting.

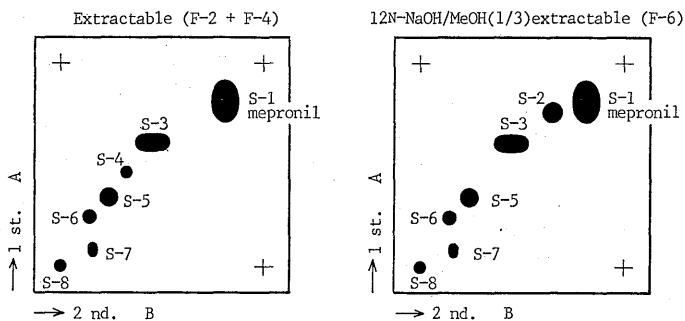
## 2. Mepronil and Its Degradation Products in Each Fractions

In both soils, the extractable fraction gradually decreased during incubation, reaching 40% after 80 days. Most of the radioactivity was mepronil, while the total degradation product was less than 8.1% (Aso, 40 days) and 9.3% (Kikugawa, 80 days) of applied radiocarbon, as shown in Table 4. The half-life of mepronil is estimated as 46 days in Aso soil and 50.5 days in Kikugawa soil. The extractable radiocarbon from bound residue fraction by treatment with 12 N-NaOH/methanol increased gradually. Again, the major portion of the released radioactivity was mepronil. Combined mepronil was 82.7% (Aso) and 93.7% (Kikugawa) after 80 days. By extrapolating the plotted curve, the half-life of total mepronil is estimated as 117 days in Aso and 120 days in Kikugawa. The location of mepronil and its degradation products in F-2

Table 4 Degradation of mepronil and formation of its degradation products in two soils.

Product No.	% of applied radiocarbon											
	Extractable					12N-NaOH/MeOH(1/3)extractable						
	Days	5	10	20	40	80	5	10	20	40	80	
Volcanic ash soil (Aso)												
F-1 + F-4	S-1 mepronil	79.2	81.7	61.4	52.8	35.9	F-6	11.1	10.0	21.3	21.6	25.1
	2	—	—	—	—	—		0.3	—	0.6	0.4	0.3
	3	—	0.7	0.7	0.6	0.4		—	—	0.1	—	—
	4	—	—	—	0.2	0.1		—	—	—	—	—
	5	—	0.6	0.4	0.4	0.3		—	0.1	—	—	0.1
	6	—	0.2	0.3	1.0	0.1		—	—	0.5	0.4	0.1
	7	—	—	—	0.2	—		—	—	—	—	—
	8	0.4	0.1	0.2	0.5	0.2		0.1	0.1	0.1	0.1	0.4
	Others	2.3	1.6	2.0	1.9	1.7		0.9	0.4	1.5	1.7	0.9
Water soluble	0.4	0.5	0.7	1.2	0.8	1.2	0.9	0.6	1.2	1.5		
Alluvial soil (Kikugawa)												
F-1 + F-4	S-1 mepronil	95.4	77.4	80.9	58.2	31.0	F-6	4.2	15.0	12.8	17.1	23.7
	2	—	—	—	—	—		—	—	—	0.1	—
	3	0.7	0.5	0.7	0.3	0.4		—	0.1	0.1	—	0.3
	4	—	—	—	0.1	0.1		—	—	—	—	—
	5	0.3	0.3	0.5	0.7	0.4		—	0.1	0.1	0.1	—
	6	0.1	0.2	0.2	0.3	0.3		—	—	—	—	0.2
	7	—	—	—	—	0.1		—	—	—	—	0.2
	8	0.2	0.2	0.2	0.4	0.6		0.1	0.1	0.2	0.1	0.3
	Others	2.4	1.8	2.2	2.4	1.8		0.2	0.8	0.6	0.7	1.8
Water soluble	0.2	0.3	0.4	1.2	0.6	0.3	0.3	0.7	1.2	2.9		

Average values of duplicate experiments.

Fig. 1 Thin-layer autoradiograms of [aniline- $U-^{14}C$ ]mepronil and its degradation products in soils.Solvent system; A: *n*-hexane/dioxane (2/1), B: benzene/methanol (19/1).

and 4, and in F-6 on a two-dimensional *tlc* is diagrammed in Fig. 1. F-3 and F-7 gave spots only at the origin.

The result of the identification and/or characterization is summarized in Table 5. As mepronil degradation products, S-1 [mepronil], S-3 [1-hydroxy-3-oxo-(3'-isopropoxyphenyl)isoindoline], S-4 [3'-isopropoxy-2-hydroxymethylbenzanilide], S-5 [3'-hydroxy-2-methylbenzanilide] and S-6 [3'-(1-hydroxymethyl-

ethoxy)-2-methylbenzanilide] were identified. An unknown spot, S-2 (UK-1), was found not in extractable but in bound residue fraction. Also, a preliminary experiment using [carbonyl- $^{14}C$ ]mepronil gave all the spots of Fig. 1 except S-2. Therefore, S-2 is a product lacking acid moiety, while S-7 (UK-2) keeps both acid and aniline moieties. However, the unknown does not seem to be important because of its small amount.

Table 5 Identification and *R<sub>f</sub>* values of degradation products in soils.

Spot No.	Identified		Solvent systems*		
	as	by	A	B	C
S-1	I	a)	0.79	0.77	0.87
2	UK-1		0.66	0.58	
3	III	b)	0.70	0.53	
4	VII	a)	0.45	0.27	0.49
5	IX	a) c)	0.36	0.22	0.25
6	X	a)	0.32	0.22	0.25
7	UK-2		0.15	0.09	
8	Unknowns		0	0	

\* A: *n*-hexane/dioxane (2/1), B: benzene/methanol (19/1), C: chloroform/ethyl acetate (5/1).

a) cochromatography, b) reduction with NaBH<sub>4</sub> and cochromatography, c) methylation with CH<sub>3</sub>N<sub>2</sub> and cochromatography.

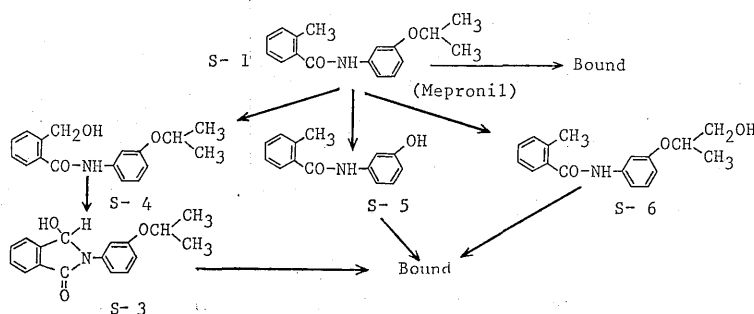


Fig. 2 Proposed degradation pathways of mepronil in soil.

Figure 2 illustrates the degradation pathways of mepronil in soil. From the chemical structure of the products, mepronil seems to be hydroxylated at three positions. The product hydroxylated at methyl group on the acid moiety (S-4) is further converted to S-3 by oxidation-cyclization. It is presumed that the intermediate (not isolated) hydroxylated at the center carbon of the isopropoxy group on the aniline moiety is unstable and degraded to S-5. Hydroxylation occurs also at the terminal carbon of the isopropoxy group giving S-6. All these products, as well as mepronil, are adsorbed on soil particles when produced. The nature of the bound residue portion unextractable with 12 N-NaOH/methanol must be further investigated.

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### 要 約

#### メプロニル (バシタック®) の湛水土壤条件下における容器内での分解

弓田隆司, 山本 出

[アニリン環-<sup>14</sup>C] メプロニルを用い, 火山灰 (阿蘇) および沖積層 (菊川) 土壤における分解の推移および主要分解生成物の同定について, 湛水条件下, 容器内試験を行なった. biometric flask 中の土壤に, 1.6 ppm でメプロニルを施用し, 80 日間暗所, 29°C にて培養し

た. メタノール: 水 (2:1) とアセトニトリルで抽出 [可溶性画分] し, さらに残渣 [土壤吸着画分] は, 12N-NaOH: メタノール (1:3) にて 70°C, 2 時間処理後, ジクロルメタンにて抽出した. メプロニルは両抽出画分での主要成分であった. 可溶性画分中のメプロニルの半減期は, 阿蘇土壤で 46 日, 菊川土壤で 50.5 日であったが, 土壤吸着画分のメプロニルを加えると, 前者は 117 日, 後者は 120 日になると推定した. 分解生成物はほとんどが土壤に吸着し, 可溶するものは少量であった. 7 種の分解生成物が確認され, そのうち 4 種: 1-hydroxy-3-oxo-(3'-isopropoxyphenyl)isoindoline, 3'-isopropoxy-2-hydroxymethylbenzanilide, 3'-hydroxy-2-methylbenzanilide および 3'-(1-hydroxymethyl)ethoxy-2-methylbenzanilide を同定した. これらは, メプロニル分子の 3 カ所で酸化を受け生じたものと推定した.